

# Historical origins and genetic diversity of wine grapes

Patrice This<sup>1</sup>, Thierry Lacombe<sup>1</sup> and Mark R. Thomas<sup>2</sup>

<sup>1</sup> INRA, UMR Diversité et Génomes des Plantes Cultivées, 2 place P. Viala, 34060 Montpellier, France

<sup>2</sup> CSIRO Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia

**The genomic resources that are available to the grapevine research community have increased enormously during the past five years, in parallel with a renewed interest in grapevine (*Vitis vinifera* L.) germplasm resources and analysis of genetic diversity in grapes. Genetic variation, either natural or induced, is invaluable for crop improvement and understanding gene function, and the same is true for the grapevine. The history and vineyard cultural practices have largely determined the genetic diversity that exists today in grapevines. In this article, we provide a synopsis of what is known about the origin and genetics of grapes and how molecular genetics is helping us understand more about this plant: its evolution, historical development, genetic diversity and potential for genetic improvement.**

## The current status of grape genomic resources

The grapevine (*Vitis vinifera* L.) is attractive for genomic research because it is diploid and has a small genome size of 475–500 Mb relative to other plants (it is approximately four times the size of *Arabidopsis* and one sixth the size of the corn genome) [1,2], consisting of 19 chromosomes. The genotypes of grape varieties are highly heterozygous and nearly all modern cultivated varieties (cultivars) are hermaphroditic, self-fertile and out-cross easily. Over the past five years there has been a rapid increase in genomic resources available for grapevine research (Box 1).

## Historical overview

The grape is unique: not only is it a major global horticulture crop but it also has ancient historical connections with the development of human culture. The main product, wine, was considered divine, a drink of the gods: even Dionysus and Bacchus were dedicated to this beverage. Other Mediterranean cultures considered that ‘the wine sprang from the blood of humans who had fought the gods’ [3] and wine has always had a major role in the way of life of Mediterranean people [4].

In the *Vitaceae* family, it is the *Vitis* genus that is of major agronomic importance. It consists of ~60 inter-fertile species that exist almost exclusively in the Northern Hemisphere (Table 1). Among them, *Vitis vinifera* is the only species extensively used in the global wine industry. It is also the only species of the genus indigenous to Eurasia and is suggested to have first appeared ~65 millions years ago

[5]. Two forms still co-exist in Eurasia and in North Africa: the cultivated form, *V. vinifera* subsp. *vinifera* (or *sativa*) and the wild form *V. vinifera* subsp. *silvestris* (or *sylvestris*), sometimes referred to as a separate subspecies. This historical separation into subspecies was based on morphological differences [6]. For the purpose of this article, we will refer to the wild-type and cultivated form as subspecies, but it could be easily argued that this distinction is not valid because any differences are most likely the result of domestication over time by humans rather than geographical isolation. Thousands of *V. vinifera* cultivars exist [7–10] but the global market for wine production is dominated by only a few cultivars owing, in large part, to how wine is currently marketed. The cultivars are generally classified according to their final production: wine grapes, table grapes and raisins. By contrast, the wild form is rare [9] and extends from Portugal to Turkmenistan, and from Rhine riversides to northern forests of Tunisia [3,9,11]. It is believed to be the ancestor of present cultivars [6] and is still observed as wild vines growing on the canopy of surrounding trees (Figure 1a). The domestication of grape seems linked to the discovery of wine, even if it is unclear which process predated the other [3,12]. During domestication, the biology of grapes underwent several dramatic changes (Figure 2) to ensure greater sugar content for better fermentation [13], greater yield and more regular production. In this process, the changes in berry and bunch size and the change from dioecious wild plants (see Glossary) to hermaphrodite cultivated plants were crucial. Changes in seed morphology also occurred [14] and even if its biological significance is unknown, this trait is used in the analysis of archaeological remains to differentiate remains of wild or cultivated grape [15,16]. It is unknown whether these changes occurred over a large period of time through sexual crosses and natural or human selection, or quickly through mutations, selection and subsequent propagation by vegetative multiplication (Box 2). Uncertainty also remains about the place and period of the original domestication and whether secondary independent domestications also occurred. Although wild grapes were probably present in many places in Europe during the Neolithic period, archaeological and historical evidence suggest that primo-domestication occurred in the Near-East. The earliest evidence of wine production was found in Iran at the Hajji Firuz Tepe site in the northern Zagros mountains circa 7400–7000 BP (before present) [3,17]. Seeds of domesticated grapes dated from ~8000 BP were also found in Georgia and in Turkey. Nevertheless, remains of seeds discovered in the Neolithic period in

Corresponding author: Thomas, M.R. (Mark.R.Thomas@csiro.au).  
Available online 26 July 2006.

## Glossary

**Ampelography:** the scientific study of grapevine identification and classification using visual descriptors, for example, leaf shape and berry characters.

**Cultivar:** a cultivated variety.

**Dioecious:** male and female reproductive structures are on different individual plants.

**Hermaphrodite:** male and female reproductive structures are on the same plant; they are in the same flower in grapevine, enabling self fertilization.

**Landrace:** old cultivar of local importance.

Western Europe also suggest exploitation of grape at this time and the remains of wild seeds were also discovered at bronze-age sites in France [14].

From the primo-domestication sites, there was gradual spread to adjacent regions such as Egypt and Lower Mesopotamia (circa 5500–5000 BP) and then further dispersal around the Mediterranean, following the main civilizations (Assyrians, Phoenicians, Greeks, Romans, Etruscans, Carthaginians) [3]. Grape cultivation reached China (2nd century) and Japan (3200 BP) [12]. Under the influence of the Romans, *V. vinifera* expanded inland and reached many temperate regions of Europe, even as far north as Germany. This expansion often followed the main trade routes (i.e. rivers such as the Rhine, Rhone, Danube and the Garonne). By the end of the Roman Empire, grape growing was common in most of the European locations where they are grown today. The Romans were the first to give names to cultivars [18] but it is difficult to relate them to modern grapes. At this time, the differentiation of table and wine grape was probably already in place in addition to the different colour types [18].

In the middle ages, the Catholic Church replaced the Romans in spreading grape cultivation to new regions and enabled the exchanged germplasm through crusades and spread of their religion through Northern Europe [12]. The extension of Islam to North Africa, Spain and Middle East also had an important role in spreading the grape (particularly table grapes). During the middle ages, the first cultivar names still used today appeared [12].

Following the Renaissance (16th century), *V. vinifera* colonized new regions (New World countries) where it

## Box 1. Genomic resources available for grapevine research

The International Grape Genome Program (IGGP) was formed in 2001 to promote international collaboration and to develop resources for the grape research community (<http://www.vitaceae.org/>). One advance since the formation of this program has been the placement of data in public databases such as NCBI.

The NCBI taxonomy web address for *V. vinifera*, the main species used for wine production, summarizes the data that have been deposited. At the time of writing, there was >228 000 nucleotide sequences, mostly ESTs, that when clustered produced a UniGene set of >15 000 genes. (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=29760&lvl=3&lin=f&keep=1&srchmode=1&unlock>)

The Institute for Genomic Research (TIGR) has produced a Grape Gene Index, by analyzing the nucleotide sequences deposited at NCBI, and Release 4.0 of the index lists >23 800 sequences as being unique. The gene ontology and metabolic pathway information for many of these sequences is also available at the TIGR site ([http://www.tigr.org/tigr-scripts/tgi/T\\_index.cgi?species=grape](http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=grape)).

Genetic maps have been produced [33,61–64] and physical maps are being produced in several laboratories [65], with a consensus map in progress. At NCBI there are >360 UniSTS (microsatellite) markers for genetic and physical mapping. Some genetic map information is also available from the Genomic-Info Research Unit (URGI) of INRA (<http://urgi.infobiogen.fr/GnpMap2/mapping/searchMap.do>).

A grape BAC library is available from the French National Resources Centre for Plant Genomics (CNRGV) (<http://cnrgv.toulouse.inra.fr/ENG/>).

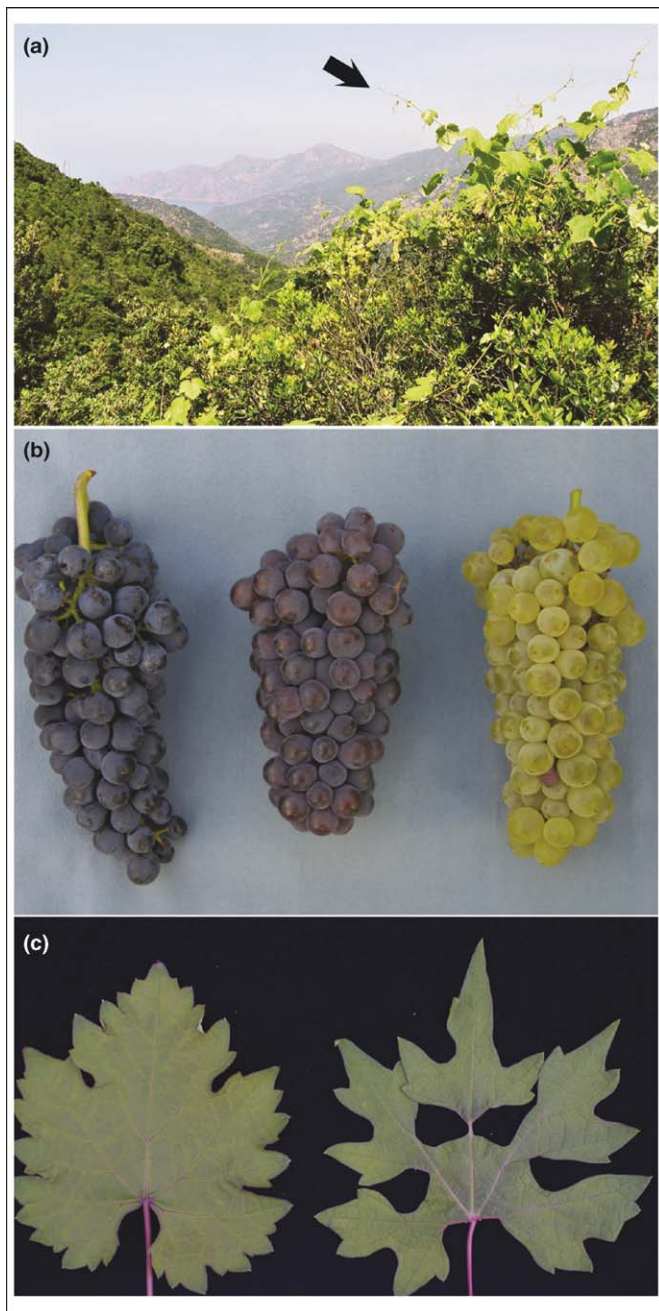
Affymetrix (<http://www.affymetrix.com/index.affx>) has released a grape array that represents 14 000 *V. vinifera* transcripts and 1700 transcripts from other *Vitis* species, which will be useful for gene expression analysis. A 14 562 70mer oligo set for investigating gene expression in grapevine is available from Qiagen (<http://www1.qiagen.com>). A project to sequence the grapevine genome by a whole-genome-sequencing approach is in progress in Italy and France ([http://www.genome.cns.fr/externe/Francais/Projects/Project\\_ML/organisme\\_ML.html](http://www.genome.cns.fr/externe/Francais/Projects/Project_ML/organisme_ML.html)).

was not indigenous. The missionaries introduced it to America, first as seeds (because they were easy to transport) and then by cuttings from their places of origin (France, German, Spain, Italy and East Europe). Cuttings were also introduced to South Africa, Australia and New Zealand in the 19th century and introduced later to North Africa [12].

**Table 1. Taxonomy of the *Vitis* genus and use of species in breeding programs during the past century<sup>a</sup>**

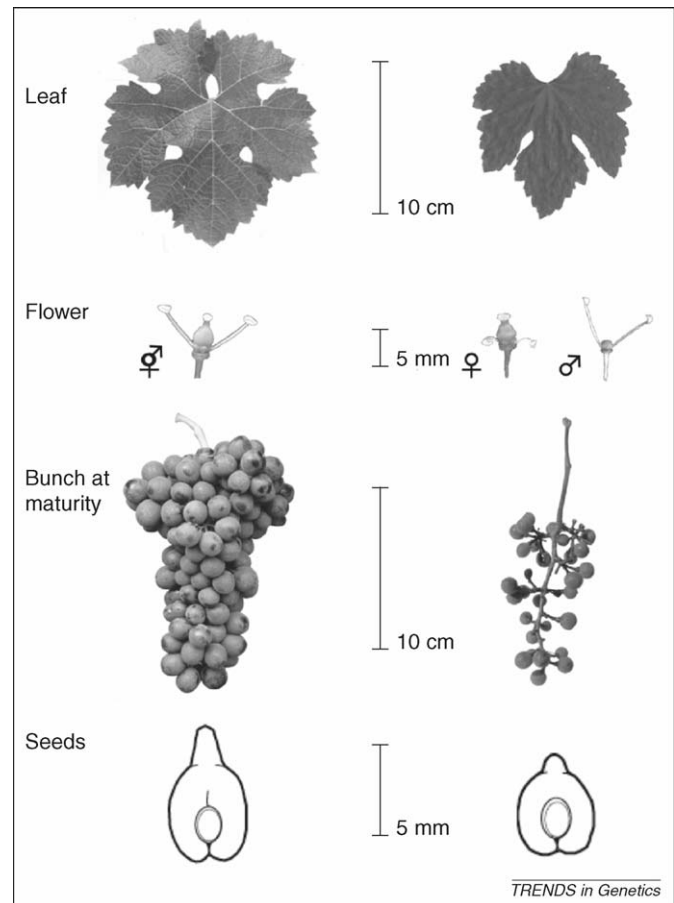
Species	Used for breeding new cultivars	Used for breeding rootstocks	Used for breeding inter-specific hybrids
<i>Muscadina rotundifolia</i>	++	+	+
<i>Vitis aestivalis</i>	–	–	++
<i>Vitis amurensis</i>	+	–	++
<i>Vitis berlandieri</i>	+	+++	–
<i>Vitis candicans</i>	–	+	–
<i>Vitis caribaea</i>	–	–	+
<i>Vitis champinii</i>	+	+	–
<i>Vitis cinerea</i>	–	+	++
<i>Vitis cordifolia</i>	–	+	+
<i>Vitis labrusca</i>	+++	++	+++
<i>Vitis longii</i>	+	++	–
<i>Vitis riparia</i>	++	+++	+++
<i>Vitis rupestris</i>	++	+++	+++
<i>Vitis simpsonii</i>	–	+	–
<i>Vitis vinifera</i>	+++++	+	++++

<sup>a</sup>The grapevine belongs to the botanical family *Vitaceae*, which consists of almost one thousand species, grouped into 17 genera. Most of which are present in the inter-tropical regions. For example, Virginia creeper (*Parthenocissus quinquefolia* and *P. tricuspidata*) are used as ornamentals in gardens, whereas plants grown indoors include *Rhoicissus* sp. and *Tetrastigma* sp. In the *Vitaceae* family, the genus *Vitis* is the only genus of agronomical importance. It consists of ~60 inter-fertile species living almost exclusively in the Northern Hemisphere, ~30 in America and 30 in Asia. Several species have been extensively used for breeding rootstocks and inter-specific hybrids particularly in the early 20th century.



**Figure 1.** Genetic diversity of grapevines. **(a)** Wild grapevines (indicated by the arrow), such as this male vine, can still occasionally be found growing on the canopy of trees in the Pyrennees in France. **(b)** Berry colour mutants of Pinot (left to right); Pinot noir (black), Pinot gris (grey), Pinot blanc (white). **(c)** Leaf mutant of Cabernet Sauvignon; the wild-type leaf is shown on the left and the mutant on the right.

At the end of the 19th century, after several millennia of geographical expansion, disease-causing agents from America reached Europe (mildews, Phylloxera) resulting in devastation and destruction of many European vineyards, drastically changing the diversity of this species. As a result, a reduction of the diversity most likely occurred for both cultivated and wild grapes. The extent of diversity of *V. vinifera* found today might be a pale reflection of what existed before the introduction of Phylloxera. European viticulture was saved from extinction by the introduction of several indigenous American, non-*vinifera*, *Vitis* species (Table 1) that were used as rootstocks and for breeding



**Figure 2.** The morphological differences between cultivated grapevines (subspecies *vinifera*) and wild grapevines (subspecies *silvestris*). The differences in the leaf, flower (male and female for *silvestris*), the bunch at maturity and the seeds are shown.

### Box 2. Genetic variation in grapevine

Three processes have had a significant impact on the development of cultivated grapevines: sexual reproduction, vegetative propagation and somatic mutations. New genotypes are produced by sexual reproduction, either by crossing or self-fertilization. Because individual grapevine plants have highly heterozygous genotypes any progeny produced from seed is a novel combination of parental alleles, resulting in phenotypic variation and segregation of traits in a progeny population. The selection of a particular phenotype, particularly a berry trait, can be a long process considering the juvenile period (three-to-five years) of grapevine plants and the additional time necessary for evaluation of a trait important for wine production. Furthermore, many generations might be necessary to recover the desired traits. Once identified, vegetative propagation (asexual) by cuttings is a method of maintaining and multiplying a highly desirable genotype so that a vineyard can be planted with a single cultivar. Cuttings are also a convenient method of transporting cultivars from one region to another. Cultivars grown today are maintained by vegetative propagation. Although clonal propagation should ensure that all plants grown from cuttings have the same genotype, the occurrence of a somatic mutation in one cutting and not other cuttings might eventually lead to plants of the same cultivar having a slightly different genotype and sometimes a different phenotype, referred to as clonal variation. This clonal variant thus appeared rapidly over one cycle of vegetative reproduction (three-to-four years). This clonal variation is more complex if the mutation is maintained in only one cell layer of the plant, resulting in genetic chimerism. If the mutation is maintained in only the L1 layer (epidermis) then it will not be passed onto progeny by sexual reproduction [51].



disease-resistant inter-specific hybrids. These inter-specific hybrids were extensively used until the middle of the 20th century (they represented ~50% of the vineyards in 1950 in France [19]), but are now rather scarce.

Over the last 50 years, the cultivated grapevine has undergone another drastic reduction of diversity, owing to the globalization of wine companies and markets, resulting in the emergence of the now familiar worldwide grown cultivars such as Chardonnay, Cabernet Sauvignon, Syrah (Shiraz) and Merlot, and the disappearance of old local cultivars or landraces (see Glossary) [13]. The sanitary selection of healthy disease-free clones has also induced a reduction in clonal diversity for these major cultivars.

Thus, the diversity of grapes existing today has been shaped by human history. Several thousand cultivars exist but most of these are largely confined to germplasm collections.

### From the wild grapevine to varieties

How did *V. vinifera* evolve from the wild to the cultivated form? As already demonstrated for many other plant species [20,21], molecular, genetic and genomic studies can help answer many questions about grape evolution and diversity. A better understanding of the exact status of the remaining wild grape populations and their relationships with existing varieties is important if we are to answer this question.

#### *Does the wild grapevine still exist today?*

Wild-growing grapevine individuals have been identified in France [22,23], Spain [24,25], Italy [26,27], Germany, Switzerland, Austria, Romania [28] and Tunisia [29], as well as many other European countries [25], but are they real *silvestris* individuals, that have never undergone cultivation, or 'escaped' individuals from vineyards or hybrids between wild and cultivated forms as described by Levadoux [9]? Genotype analysis could answer this.

Recent analyses focused on the use of microsatellite (SSR) and more recently on single nucleotide polymorphism (SNP) markers [22,23,25,28,30–32] have shown clear distinctions between wild and cultivated individuals. Only one report [31] showed a close affinity between wild samples and cultivars. These divergent results could be due to the differences in sample size and composition between the studies, or the uncertainty in classifying the wild growing individuals. Morphological traits of the plant and seeds are important for the characterization of true *silvestris* types (Figure 2). Approximately 250 putative *silvestris* individuals from France are currently being analyzed using 20 well-scattered microsatellite markers selected on a molecular map of *V. vinifera* [33]. This study should help establish whether true wild individuals with no genetic contribution from cultivated compartment still exist in France. Only when extensive molecular data from wild individuals and cultivars from different regions becomes available will we be able to draw a clearer picture about the existence of *silvestris*-type individuals in Europe. Analysis of wild grapes from eastern counties such as Turkey, Iran or Georgia, the presumed centre of primo-domestication, will be fundamental in this respect because it might help elucidate the diversity of the grapevine genetic pool used

for domestication and identify the main events that enabled the morphological transformation from the wild form to cultivated vinifera.

The wild ancestors and current cultivars differ in several traits, for example, sugar content, flower sex, berry and bunch size. The analysis of allelic diversity for genes involved in these traits would be of great value for the analysis of wild individuals and the definition of their status. Furthermore, as demonstrated for maize [34], the comparison of the level of genetic diversity between the wild ancestor and the cultivated forms could enable the identification of regions of the genome that have undergone a strong selection during the domestication process, and thus identify genes controlling such traits.

#### *Early domestication and propagation by seed*

Sexual reproduction and planting of seeds seems to have had an important early role in the domestication and expansion of viticulture into new regions. Seeds are believed to have been the preferred way of long-range transportation of cultivars as suggested by archaeological remains [35]. More direct evidence can now be obtained by DNA analysis and the identification of parentages [36,37]. These parentage studies demonstrate the importance of sexual crosses in the past for the generation of new phenotypes and the adoption and spread, by vegetative propagation (cuttings), of specific genotypes with desirable characters. The Pinot and Gouais varieties of grape were successful in creating progeny [36] that were maintained by viticulturalists of the period and many of these progeny are cultivated in the north east of France and are well-adapted to this environment. However, only one example of a direct relationship between a wild and cultivated individual has been published [31], suggesting the absence of gene flux between wild and cultivated compartments. The flowering times of wild individuals is different from the flowering times of the cultivated forms (Lacombe *et al.*, personal communication), reducing the possibility of gene flow through pollen. Characterization of seeds obtained through open-pollinization of wild female plants is underway [Di Vecchi *et al.*, unpublished] and will give direct evidence of pollen flux between compartments in areas where they co-exist.

#### *Was there a single domestication event or many domestication events?*

Although many studies of genetic diversity have already been published [37], few studies examine a large geographical area that would provide evidence for the existence of diverse domestication pools or a single major domestication event followed by selection of diverse genotypes. An appreciable level of genetic differentiation between table and wine cultivars has been detected using microsatellite markers [31], with Muscat types also somewhat distinct. These differences could be due to divergent selection for berry size and the use of the oriental gene pool for the development of large berry and large cluster table cultivars, and divergent selection for the Muscat flavour for Muscat types [38]. Differences have also been detected between European wine cultivars of those from different regions [39], and the possibility of two independent

domestication events (one in the Near East, one in Western Europe) was shown more recently using chloroplast microsatellite markers [25]. However, this finding was contradicted by Aradhya *et al.* [31], who found most divergence within groups of cultivars and narrow differentiation between different groups, concluding that there might have been a single complex gene pool with current varieties arising by strong artificial selection. Two limitations however influence this type of analysis. First, a strong geographical genetic structure would imply limited exchange of genetic material between regions and is most unlikely for grapevine, where spread of genetic material between regions has acted to homogenize the pools over time. Chardonnay, often referred to as a French cultivar, is a good example, being the result of a cross between Pinot and Gouais (also called Heunisch weiss) of Croatian origin [36]. Second, because of this exchange of material between regions, the geographical origin of cultivars is not easy to establish. If a French origin of Cabernet Sauvignon is recognized (which is strongly supported by the parentage of this cultivar [40]), the origin of other cultivars is less certain and will probably continue to evolve with new data. Syrah was thought to be of eastern origin [38], but its parentage determined by DNA profiling is now thought to be French [41]. Therefore, deeper sampling from different geographical regions is needed, with concise, accurate information on the geographical and genetic origins of the genotypes and the study of haplotypes rather than genotypes.

It must also be recognized that the origin of many cultivars might not be resolved because of the extinction of the parent. Pinot noir, for example, is sometimes referred to as 'archaic' and could have been present in the 1st century, with the Pinot name first recorded in the

14th century [9]. To date, no ancestors have been discovered for this cultivar despite its importance. Does this suggest that Pinot is not too far genetically removed from a wild grapevine or that in the distant past there was a genetic bottleneck with only a few ancient varieties surviving? The numerous progeny of Pinot and Gouais identified (>275 cultivars [42] M. DiVecchi *et al.*, unpublished) is a strong indication that there was a historical genetic bottleneck in some regions, possibly owing to cultivation practices and/or ownership of the vineyards.

#### *How old are modern grape varieties?*

Speculation about the historical origins of current cultivars is common in popular consumer wine publications; but is there any evidence that some cultivars that were created during antiquity or the middle ages still exist today as a result of vegetative propagation?

The analysis of ancient plant DNA has made important advances in recent years [43]. DNA of grape seeds between 2600 and 1700 years of age have been successfully analyzed using a few microsatellite markers [44], and this opens the way for analysis of archaeological grape samples. Because seeds could be the result of crosses, analyses on ancient wood remains would be needed to characterize the identity of these old cultivars and compare their DNA profile with those from modern cultivars. Nevertheless, a combination of historical and molecular data can still be useful. For example, the Mission cultivar is grown in many South American countries and was probably introduced into South America by the Spanish missionaries as seeds [19]. The analysis of its DNA and that of many South American cultivars revealed that many of the latter were synonyms and siblings of the Mission variety (Table 2). It is therefore tempting to suggest that the Mission cultivar introduced into South America in

**Table 2. Microsatellite analysis of the Mission cultivar and its relationship with other cultivars from South America and Spain<sup>a</sup>**

Synonyms of the cultivar mission identified using 20 markers							
Cultivar name	Origin and use						
Mission	Mexico, California, W, n						
País	Chile, W, n						
Rosa del Peru	Peru, W, n						
Negra corriente	Peru, W, n						
Criolla chica	Argentina, W, n						
Proposed relationships between the cultivar mission and varieties from Spain and south America							
Cultivar name	Origin	Use and colour	LOD <sup>b</sup>	Relationship to mission	Parent 1	Parent 2	LOD <sup>c</sup>
Perruno	Spain	W, b	32.8	Progeny or sibling <sup>d</sup>			
Torrontès sanjuanino	Argentina, Chile, Peru	T, W, b	33.54	Progeny	Mission	Muscat d'Alexandrie B	63.15
Quebranta	Peru	T, n	32.02	Progeny	Mission	Negra mole N (Spain)	58.67
Torrontès riojano	Argentina	T, b	31.99	Progeny	Mission	Muscat d'Alexandrie B	67.08
Jaen negro	Spain	W, n	30.16	Progeny	Mission	Jaen B (Spain)	61.46
Criolla San Juanina	Argentina	T, W, n	29.49	Progeny	Mission	Muscat d'Alexandrie B	69.03
Cereza	Argentina, Uruguay	T, W, n	27.7	Progeny	Mission	Muscat d'Alexandrie B	69.12

<sup>a</sup>Abbreviations: LOD, log of the odds (lod score); T, table; W, wine; b, white; n, black; B, white.

<sup>b</sup>Lod score of single parentage with Mission.

<sup>c</sup>Lod score for parent couples.

<sup>d</sup>Perruno and Mission share at least one allele at each locus.

the 16th century was vegetatively propagated and transported by cuttings to different American countries where it was renamed according to locality. Similarly, in Europe, Chardonnay can be traced back to the middle ages and is the result of a cross between Pinot noir and Gouais [36], and thus these three cultivars have been maintained by vegetative propagation since at least the middle ages. These examples demonstrate that vegetative propagation of grapevine cultivars, to create new vineyards and move viticulture from one region to another, has been performed for many centuries.

Despite the importance of vegetative propagation in maintaining cultivars in an almost identical state, recent reports of genetic characterization of existing cultivars have shown that mutations are frequent in grapevines and have had a role in generating genetic diversity.

#### *The role of mutations*

Both sexual crossing and natural mutations have been the drivers during grapevine evolution. One of the most important traits in the domestication of grapevine, the appearance of hermaphrodite flowers, seems to be the result of a mutation [45]. It is not known when this form was first selected and used by humans but because it removed the need to maintain both male and female plants the adoption would have been rapid.

Because much of grapevine propagation is performed through cuttings, mutations can accumulate over time and lead to morphological and agronomical differences, and thus the creation of new cultivars.

Many examples of transposon and retrotransposon-based mutations have been published in plants; grapes are no exception and several elements have been identified to date [46,47]. Recently, the insertion of a gypsy-type retroelement (*Gret1*) in the promoter region of a regulatory gene of the Myb family has been shown to cause the loss of black berry skin colour in homozygous individuals [48]. The original wild grapes are believed to have had black berries [12] and plants with white berries were probably selected and maintained during the domestication process.

The degree of SNP polymorphism is not well known in grapevines. It has recently been reported as being extensive in genes (a mean frequency of one SNP every 78 bp between species and every 119 bp in *V. vinifera* [49]) but more accurate data for many regions of the genome are needed. It has been shown that a single nucleotide mutation in the *VuGAI* gene in grapevine not only has an effect on the number of leaf hairs but also reduces the stature of the plant and promotes flowering [50]. The original mutant plant, Pinot Meunier, was a chimera that had a hairy leaf phenotype, with the other phenotypes appearing only after regeneration of plants from the separate cell layers (L1 and L2) [51].

Stable chimeric plants result from somatic mutations if the mutation occurs in a cell of the shoot apical meristem and the mutated cells dominate one cell layer over a period of time. This has previously been discussed in relation to genetic improvement, where depending on which cell layer the mutation occurs, the mutation might be passed onto the next generation [51]. The occurrence of this phenomenon and the maintenance of mutations are due,

in a large part, to the age of many grapevine cultivars that have accumulated mutations over time and to the annual pruning of shoots and extensive vegetative propagation that assists in separating mutant cells from wild-type cells. Natural mutants that have leaf, flower or berry changes have been selected in this way (Figures 1b,c). The Pinot family has been particularly well analyzed for vegetative, floral and berry mutants. Pinot noir is the original variety with a black berry, Pinot gris is the grey berry form, thought to be a chimera with a mutation for berry colour in one cell layer, and Pinot blanc is the white berry form, thought to have the mutation in both cell layers (Figure 1b).

Sometimes, independent mutations can lead to identical phenotypes, as suggested for the seedless phenotypes in Sultanina (Sultana, Thompson Seedless), Emperor Seedless and Chasselas apyrène [52]. Humans have selected this mutant seedless phenotype for table grape.

It is now known that non-visual mutations are also present and maintained in grapevine cultivars. Mutations in microsatellite markers have been described and the implications of this for cultivar identity and ancestry determination have been discussed elsewhere [51]. Many of these microsatellite mutations have been shown to exist in a chimeric state with only one cell layer having the mutation [51,53,54].

#### **V. *vinifera* germplasm and genetic diversity**

Molecular evidence shows that both sexual and asexual multiplication and mutations have had a major role in the expansion and diversification of grapevine. The number of different varieties held in germplasm collections around the world is estimated at ~10 000 [7]. Nearly every wine-growing country has its own grapevine germplasm collection, owing to quarantine restrictions and the need to maintain the material in the field as living plants. As already mentioned for Mission cultivar, many names for the same material (synonyms) exist, but because of some peculiar features of the varieties or of the regions where they were grown, a convergence of names for different cultivars (homonyms) has also occurred (Table 3). Microsatellite markers in the early 1990s were first shown to be useful for determining cultivar identity and parentage, and for identifying mistakes [55,56]. Many microsatellite marker studies have since been published identifying synonyms and homonyms that were either previously suspected by ampelography studies or unknown [37,64]. The identification of these 'onyms' will also help to determine the true extent of genetic diversity. Based on DNA profiling results, we can speculate that a more accurate estimate of variety numbers might be closer to 5000 varieties with many of them being closely related.

Characterization of diverse germplasm collections using microsatellite markers have been achieved [31,39,57,58] but the cross correlation between all these studies remains to be performed. A coding strategy has been proposed to compare the data between laboratories easily [59]. Reference data for six microsatellite markers and a set of internationally recognized cultivars and rootstocks are now available at <http://www.montpellier.inra.fr/vassal>. Additional reference information and tools for the

**Table 3. Examples of synonyms, homonyms and mutant forms of popular cultivars<sup>a</sup>**

Official French name	Main synonyms (country)	Secondary synonyms	Known mutations <sup>b</sup>	Homonyms
Cabernet Sauvignon	-	Bidure, Petite Vidure, Bouchet (FRA); Petit Cabernet (MAR)	Bronze berry form; White berry form	Cabernet franc (FRA) Cabernet Suintory (JPN) Pfeffer Cabernet (USA) Petite Syrah Durif (USA)
Syrah	Shiraz, Hermitage (AUS)	Serine	-	
Sultanine	Sultana (AUS, ZAF) Sultanina (Mc) Thompson seedless (USA) Keshmish (Mc)		Sultanine rose Gora Chirine	
Grenache	Garnacha tinta (ESP, Mc); Tokay rosso, Cannonau (ITA); Alicante (ESP)	Roussillon, Bois jaune(FRA); Lladoner, Gironet (ESP); Francese (ITA); Abundante (PRT)	Grenache blanc Grenache gris Ledoner pelut	Garnacha tintorera; Alicante Bouschet (ESP)
Pinot noir	Blauer Spatburgunder (Mc); Burgundske Modre (CEZ)		Pinot gris Pinot blanc Pinot meunier Pinot teinturier	Pineau de la Loire (USA); Chenin Pineau d'Aunis (FRA); Pinot Chardonnay (Mc); Chardonnay Pinot Saint-Georges (USA); Négrette Pinotage (ZAF)

<sup>a</sup>Abbreviations: AUS, Australia; CEZ, Czech Republic; ESP, Spain; FRA, France; ITA, Italy; JPN, Japan; MAR, Morocco; Mc, Many countries; PRT, Portugal; USA, United States of America; ZAF, South Africa.

<sup>b</sup>The mutation induces a change in the characteristics of the berry and wine and the mutants are often identified as different cultivars.

automatic coding of the data are being developed (P. This *et al.*, unpublished). This should assist in the development of an international database (<http://www.genres.de/eccdb/vitis>) [55] that will enable the scientific community to estimate the extent of diversity in *Vitis* and *V. vinifera*. It is expected that grapevine genomic studies will increasingly depend on well-characterized unrelated genetic resources through the definition of core-collections. Material in these core collections can then be used for creating genetic mapping populations from defined parents and for linkage disequilibrium [60] and genetic association studies.

Somatic mutations combined with vegetative propagation have had a major role in increasing the genetic diversity in grapevine and the use of these mutants in genomic studies will help assign function and roles to specific genes. The identification and maintenance of these mutants in germplasm collections is of crucial importance; the major grapevine germplasm collection in France at Vassal has been engaged in this process for many years, and currently contains >200 grapevine mutants.

### Concluding remarks

Further research is required to determine accurately the extent and origin of existing genetic diversity, which is mostly confined to germplasm collections. A real possibility exists that some of these germplasm collections will be reduced in size, owing to dwindling resources, and many unique genotypes might be lost. Extensive DNA profiling of the grape varieties found in collections and the development of a common database would seem to be a priority to determine the true number of varieties and the relationships, genetic diversity and identification of unique individuals in these collections. Such data would also assist in historical studies investigating domestication events. Wild

grapevines are still poorly characterized. More extensive and combined analyses of wild individuals from a broad geographical area are crucial for understanding the role of *V. vinifera silvestris* in the domestication process. Until now, most DNA profiling studies in grapevine have been performed using neutral markers (such as non-coding microsatellite markers) but the availability of numerous grapevine ESTs and the increasing characterization of genes should encourage the analysis of allelic diversity in genes involved in the genetic control of important traits. This would not only help us understand the biology of the grape but would have direct application for crop improvement.

### Acknowledgements

This work was supported in part by CSIRO Plant Industry, the Commonwealth Cooperative Research Centre Program, specifically the Cooperative Research Centre for Viticulture (CRCV) and the Grape and Wine Research and Development Cooperation (GWRDC), INRA and the French Ministry of Research and Agriculture. We thank J.P. Bruno for assistance with Figure 2.

### References

- 1 Thomas, M.R. *et al.* (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. *Theor. Appl. Genet.* 86, 173–180
- 2 Lodhi, M.A. and Reisch, B.I. (1995) In situ hybridization in *Vitis vinifera* L. *Theor. Appl. Genet.* 90, 11–16
- 3 McGovern, P.E. (2004) *Ancient wine: the search for the origins of viticulture*. Princeton University Press
- 4 Blanco, J.L. (1997) La plus vieille histoire du vin. In *L'Histoire du vin, une histoire de rites* (Office International de la Vigne et du Vin, eds), pp. 31–41, Salomon
- 5 de Saporta, G. (1879) *Le monde des plantes avant l'apparition de l'homme*. Masson
- 6 Zohary, D. (1995) Domestication of the Grapevine *Vitis vinifera* L. in the Near East. In *The origins and Ancient History of Wine* (Mc Govern, P.E. *et al.*, eds), pp. 23–30, Gordon and Breach



- 7 Alleweldt, G. and Dettweiler, E. (1994) *The Genetic Resources of Vitis: World List of Grapevine Collections*. (2nd edn), Geilweilerhof
- 8 Galet, P. (2000) *Dictionnaire Encyclopédique des Cépages*. Hachette
- 9 Levadoux, L. (1956) Les populations sauvages et cultivées de *Vitis vinifera* L. *Ann. Amélior. Plantes* 6, 59–117
- 10 Viala, P. and Vermorel, V. (1901–09) *Ampelographie. Traité général de viticulture* Vol. 1–7, Masson
- 11 Arnold, C. *et al.* (1998) Situation de la vigne sauvage *Vitis vinifera* subsp. *silvestris* en Europe. *Vitis* 37, 159–170
- 12 Royer, C. (1988) Mouvement historiques de la vigne dans le monde. In *La Vigne et le Vin* (La Manufacture et la Cité des sciences et de l'industrie, eds), pp. 15–25, Graficas
- 13 Pouget, R. (1988) *Vitis vinifera*, histoire et évolution. In *La Vigne et le Vin* (La Manufacture et la Cité des sciences et de l'industrie, eds), pp. 15–25, Graficas
- 14 Marinval, P. (1997) Vigne sauvage et Vigne cultivée dans le Bassin méditerranéen: Emergence de la viticulture - Contribution archéobotanique. In *L'Histoire du Vin, une Histoire de Rites* (Office International de la Vigne et du Vin, eds), pp. 137–172, Salomon
- 15 Terral, J.-F. (2002) Quantitative anatomical criteria for discriminating wild grapevine (*Vitis vinifera* ssp. *silvestris*) from cultivated vines (*Vitis vinifera* ssp. *vinifera*). *British Archaeological Reports, International Series* 1063, 59–64
- 16 Reish, B.I. and Pratt, C. (1996) Grapes. In *Fruit Breeding, Volume II, Vine and Small Fruits* (Janik, J. and Moore, J.N., eds), pp. 297–369, John Wiley and Sons
- 17 McGovern, P.E. *et al.* (1996) Neolithic resinated wine. *Nature* 381, 480–481
- 18 Roxas, C. (1814) *Essai sur les variétés de la vigne qui végètent en Andalousie*. Imprimerie Poulet, Paris
- 19 Galet, P. (1957) *Cépages et Vignobles de France*. Le Paysan du Midi
- 20 Harris, S.A. *et al.* (2002) Genetic clues to the origin of the apple. *Trends Genet.* 18, 426–430
- 21 Moore, G.A. (2001) Oranges and lemons: clues to the taxonomy of citrus from molecular markers. *Trends Genet.* 17, 536–540
- 22 This, P. *et al.* (2001) Caractérisation de la diversité d'une population de vignes sauvages du Pic Saint-Loup (Hérault) et relations avec le compartiment cultivé. *Genet. Selec. Evol.* 33, 289–304
- 23 Lacombe, T. *et al.* (2002) Contribution à la caractérisation et à la protection in situ des populations de *Vitis vinifera* L. ssp. *silvestris* (Gmelin) Hegi, en France. In *Les Actes du BRG no 4: Genetic Inheritance, the Diversity and the Resources* (BRG, eds), pp 381–404, Bialec.
- 24 Benitez, L. and Ocete Rubio, R. (1992) Presencia de *Vitis vinifera silvestris* en la serrania de Grazailem: algunas consideraciones fitosanitarias. *VitiViticultura* 11–12, 33–39
- 25 Arroyo-Garcia, R. *et al.* Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. *Molecular Ecology* (in press)
- 26 Anzani, R. *et al.* (1990) Wild grapevine (*Vitis vinifera* var. *silvestris*) in Italy: diffusion, characteristics and germplasm preservation, 1989 report. In *Proceeding of the 5th International Symposium on Grape Breeding. Vitis* (Special issue), 97–113
- 27 Grando, M.S. *et al.* (1995) RAPD markers in wild and cultivated *Vitis vinifera*. *Vitis* 34, 37–39
- 28 Grassi, F. *et al.* (2003) Evidence of a secondary grapevine domestication centre detected by SSR analysis. *Theor. Appl. Genet.* 107, 1315–1320
- 29 Perret, M. *et al.* (1998) Relationship and genetic diversity of wild and cultivated grapevines (*Vitis vinifera* L.) in central Europe based on microsatellite markers. *Acta Hort.* 528, 155–159
- 30 Snoussi, H. *et al.* (2004) Genetic relationship among cultivated and wild grapevine accessions from Tunisia. *Genome* 47, 1211–1219
- 31 Aradhya, M.K. *et al.* (2003) Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genet. Res.* 81, 179–192
- 32 Di Vecchi Staraz M. *et al.* (2004) Studio sulle relazioni genetiche tra viti selvatiche e cultivate in Toscana. In *Proceedings of the 2nd International Symposium on Sangiovese* (ARISA, eds), Firenze.
- 33 Adam-Blondon, A.-F. *et al.* (2004) Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. *Theor. Appl. Genet.* 109, 1017–1027
- 34 Wright, S.I. *et al.* (2005) The effects of artificial selection on the maize genome. *Science* 308, 1310–1314
- 35 Long, L. (1987) Les épaves du Grand-Congloué: étude du journal de fouille de Fernand Benoit. *Archéonautica* 7, 9–36
- 36 Bowers, J. *et al.* (1999) Historical genetics: the parentage of Chardonnay, Gamay, and other wine grapes of northeastern France. *Science* 285, 1562–1565
- 37 Sefc, K.M. *et al.* (2001) Microsatellite markers for grapevine: a state of art. In *Molecular Biology & Biotechnology of Grapevine* (Roubelakis-Angelakis, K.A., eds), pp. 433–463, Kluwer Academic Publishers.
- 38 Bronner, A. (2003) *Muscat et variétés Muscatées*. INRA Editions
- 39 Sefc, K.M. *et al.* (2000) Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theor. Appl. Genet.* 100, 498–505
- 40 Bowers, J.E. and Meredith, C.P. (1997) The parentage of a classic wine grape. Cabernet Sauvignon. *Nat. Genet.* 16, 84–87
- 41 Bowers, J.E. *et al.* (1998) A single pair of parents proposed for a group of grapevine varieties in northeastern France. *Acta Hort.* 528, 129–133
- 42 Boursiquot, J.M. *et al.* (2004) Le Gouais, un cépage clé du patrimoine viticole Européen. *Bull. OIV* 875–876, 5–19
- 43 Parducci, L. and Petit, R.J. (2004) Ancient DNA - unlocking plants' fossil secrets. *New Phytol.* 161, 335–339
- 44 Manen, J.F. *et al.* (2003) Microsatellites from archaeological *Vitis vinifera* seeds allow a tentative assignment of the geographical origin of ancient cultivars. *J. Archaeo. Sci.* 30, 721–729
- 45 Doazan, J.-P. and Rives, M. (1967) Sur le déterminisme génétique de sexe dans le genre *Vitis*. *Ann. Amélior. Plantes* 17, 105–111
- 46 Verries, C. *et al.* (2000) Cloning and characterization of *Vine-1*, a LTR retrotransposon like element in *Vitis vinifera* L. and other *Vitis* species. *Genome* 43, 366–376
- 47 Pelsy, F. and Merdinoglu, D. (2002) The complete sequence of *tvv1* a family of Ty1 copia-like retrotransposon of *Vitis vinifera* L.; reconstructed by chromosome walking. *Theor. Appl. Genet.* 105, 614–621
- 48 Kobayashi, S. *et al.* (2004) Retrotransposon-induced mutations in grape skin color. *Science* 304, 982
- 49 Salmaso, M. *et al.* (2004) Genome diversity and gene haplotypes in the grapevine (*Vitis vinifera* L.), as revealed by single nucleotide polymorphisms. *Mol. Breed.* 14, 385–395
- 50 Boss, P.K. and Thomas, M.R. (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* 416, 847–850
- 51 Franks, T. *et al.* (2002) Chimerism in grapevines: implications for cultivar identity, ancestry and genetic improvement. *Theor. Appl. Genet.* 104, 192–199
- 52 Adam-Blondon, A.-F. *et al.* (2001) Assessment of the usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. *Vitis* 40, 147–156
- 53 Hocquigny, S. *et al.* (2004) Diversification within grapevine cultivars goes through chimeric states. *Genome* 47, 579–589
- 54 Riaz, S. *et al.* (2002) Genetic divergence and chimerism within ancient asexually propagated winegrape cultivars. *J. Am. Soc. Hort. Sci.* 127, 508–551
- 55 Thomas, M.R. *et al.* (1994) DNA typing of grapevines: a universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Mol. Biol.* 25, 939–949
- 56 Thomas, M.R. and Scott, N.S. (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theor. Appl. Genet.* 86, 985–990
- 57 Martin, J.P. *et al.* (2003) Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. *Genome* 46, 10–18
- 58 Lopes, M.S. *et al.* (1999) The use of microsatellites for germplasm management in a Portuguese grapevine collection. *Theor. Appl. Genet.* 99, 733–739
- 59 This, P. *et al.* (2004) Development of a common set of standard varieties and standardized method of scoring microsatellites markers for the analysis of grapevine genetic resources. *Theor. Appl. Genet.* 109, 1448–1458



- 60 Barnaud, A. *et al.* (2006) Linkage disequilibrium in cultivated grapevine, *Vitis vinifera* L. *Theor. Appl. Genet.* 112, 708–716
- 61 Fischer, B.M. *et al.* (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theor. Appl. Genet.* 108, 501–515
- 62 Grando, M.S. *et al.* (2003) Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Mchx. *Theor. Appl. Genet.* 106, 1213–1224
- 63 Lodhi, M.A. *et al.* (1995) A molecular marker based linkage map of *Vitis. Genome* 38, 786–794
- 64 Riaz, S. *et al.* (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. *Theor. Appl. Genet.* 108, 864–872
- 65 Adam-Blondon, A.-F. *et al.* (2005) Construction and characterization of BAC libraries from major grapevine cultivars. *Theor. Appl. Genet.* 110, 1363–1371

## Five things you might not know about Elsevier

### 1.

Elsevier is a founder member of the WHO's HINARI and AGORA initiatives, which enable the world's poorest countries to gain free access to scientific literature. More than 1000 journals, including the *Trends* and *Current Opinion* collections and *Drug Discovery Today*, are now available free of charge or at significantly reduced prices.

### 2.

The online archive of Elsevier's premier Cell Press journal collection became freely available in January 2005. Free access to the recent archive, including *Cell*, *Neuron*, *Immunity* and *Current Biology*, is available on ScienceDirect and the Cell Press journal sites 12 months after articles are first published.

### 3.

Have you contributed to an Elsevier journal, book or series? Did you know that all our authors are entitled to a 30% discount on books and stand-alone CDs when ordered directly from us? For more information, call our sales offices:

+1 800 782 4927 (USA) or +1 800 460 3110 (Canada, South and Central America)  
or +44 (0)1865 474 010 (all other countries)

### 4.

Elsevier has a long tradition of liberal copyright policies and for many years has permitted both the posting of preprints on public servers and the posting of final articles on internal servers. Now, Elsevier has extended its author posting policy to allow authors to post the final text version of their articles free of charge on their personal websites and institutional repositories or websites.

### 5.

The Elsevier Foundation is a knowledge-centered foundation that makes grants and contributions throughout the world. A reflection of our culturally rich global organization, the Foundation has, for example, funded the setting up of a video library to educate for children in Philadelphia, provided storybooks to children in Cape Town, sponsored the creation of the Stanley L. Robbins Visiting Professorship at Brigham and Women's Hospital, and given funding to the 3rd International Conference on Children's Health and the Environment.

## Endeavour



Coming soon in the quarterly magazine for the history and philosophy of science:

**Disputed discovery: vivisection and experiment in the 19th century** by C. Berkowitz  
**Engineering fame: Isambard Kingdom Brunel** by P. Fara  
**'But man can do his duty': Charles Darwin's Christian belief** by J. van der Heide



**Endeavour is available on ScienceDirect, [www.sciencedirect.com](http://www.sciencedirect.com)**